

WHAT IS CLAIMED IS:

1. A modified DNA polymerase which during DNA sequencing effectively incorporates fluorescent dye-labeled dideoxynucleotide terminators ddCTP, ddATP, ddTTP and ddGTP, and their analogs, and reduces selective discrimination against incorporation of fluorescent dye-labeled dideoxynucleotide terminators ddCTP and ddATP,

wherein the DNA polymerase in its unmodified state selectively discriminates against incorporation of fluorescent dye-labeled dideoxynucleotide terminators ddCTP and ddATP but does not discriminate against incorporation of fluorescent dye-labeled dideoxynucleotide terminators ddTTP and ddGTP.

2. The DNA polymerase according to claim 1 which is a modified *Bacillus stearothermophilus* DNA polymerase.

3. The DNA polymerase according to claim 2 which has an amino acid sequence that shares not less than 95% homology of a DNA polymerase isolated from a strain of *Bacillus stearothermophilus*, *Bacillus caldotenax* or *Bacillus caldolyticus*.

4. The DNA polymerase according to claim 1 wherein the DNA polymerase is a modified DNA polymerase obtained from a mesophilic bacterium.

5. The DNA polymerase according to claim 1 which is a thermostable DNA polymerase having proofreading 3'-5' exonuclease activity during DNA sequencing of a DNA strand from a

template, such that the DNA polymerase functions to excise mismatched nucleotides from the 3' terminus of the DNA strand at a faster rate than the rate at which the DNA polymerase functions to remove nucleotides matched correctly with nucleotides of the template.

6. A modified *Bacillus*

*stearotherophilus* DNA polymerase which during DNA sequencing effectively incorporates fluorescent dye-labeled dideoxynucleotide terminators ddCTP, ddATP, ddTTP and ddGTP, and reduces selective discrimination against incorporation of fluorescent dye-labeled dideoxynucleotide terminators ddCTP and ddATP,

wherein the DNA polymerase in its unmodified state selectively discriminates against incorporation of fluorescent dye-labeled dideoxynucleotide terminators ddCTP and ddATP but does not discriminate against incorporation of fluorescent dye-labeled dideoxynucleotide terminators ddTTP and ddGTP,

wherein the DNA polymerase has proofreading 3'-5' exonuclease activity during DNA sequencing of a DNA strand from a template, such that the DNA polymerase functions to excise mismatched nucleotides from the 3' terminus of the DNA strand at a faster rate than the rate at which the DNA polymerase functions to remove nucleotides matched correctly with nucleotides of the template.

7. The DNA polymerase according to claim 6, which has the amino acid sequence SEQ ID NO:4.

8. The DNA polymerase according to claim 6, which is encoded by a DNA segment having

the nucleotide sequence of SEQ ID:NO 3.

9. A host cell which produces a modified DNA polymerase which during DNA sequencing effectively incorporates fluorescent dye-labeled dideoxynucleotide terminators ddCTP, ddATP, ddTTP and ddGTP, and reduces selective discrimination against incorporation of fluorescent dye-labeled dideoxynucleotide terminators ddCTP and ddATP,

wherein the DNA polymerase in its unmodified state selectively discriminates against incorporation of fluorescent dye-labeled dideoxynucleotide terminators ddCTP and ddATP but does not discriminate against incorporation of fluorescent dye-labeled dideoxynucleotide terminators ddTTP and ddGTP.

10. The host cell according to claim 9, wherein the modified DNA polymerase has proofreading 3'-5' exonuclease activity during DNA sequencing of a DNA strand from a template, such that the DNA polymerase functions to excise mismatched nucleotides from the 3' terminus of the DNA strand at a faster rate than the rate at which the DNA polymerase functions to remove nucleotides matched correctly with nucleotides of the template.

11. The host cell according to claim 9, which produces a DNA polymerase having the amino acid sequence SEQ ID NO:4.

12. The host cell according to claim 9, wherein the DNA polymerase is encoded by the nucleotide sequence SEQ ID NO:3.

13. The host cell of claim 9, which is E. coli.

14. A method of sequencing a DNA strand comprising the steps of

i) hybridizing a primer to a DNA template to be sequenced;

ii) extending the primer using the modified DNA polymerase of claim 1, in the presence of adequate amounts of nucleotide bases dATP, dGTP, dCTP and dTTP, or their analogs, and fluorescent dye-labeled dideoxynucleotide terminators,

under such conditions that the DNA strand is sequenced.

15. The method according to claim 14, wherein the DNA polymerase is a modified *Bacillus stearothermophilus* DNA polymerase.

16. The method according to claim 14, wherein the modified DNA polymerase has an amino acid sequence that shares not less than 95% homology of a DNA polymerase isolated from a strain of *Bacillus stearothermophilus*, *Bacillus caldotenax* or *Bacillus caldolyticus*.

17. The method according to claim 14, wherein the modified DNA polymerase has the amino acid sequence SEQ ID NO:4.

18. The method according to claim 14, wherein the DNA polymerase is encoded by the nucleotide sequence SEQ ID NO:3.

19. The method according to claim 14, wherein the DNA polymerase is a modified DNA polymerase obtained from a mesophilic bacterium.

20. The method according to claim 14, wherein the DNA polymerase is a thermostable DNA polymerase having proofreading 3'-5' exonuclease activity during DNA sequencing of a DNA strand from a template, such that the DNA polymerase functions to excise mismatched nucleotides from the 3' terminus of the DNA strand at a faster rate than the rate at which the DNA polymerase functions to remove nucleotides matched correctly with nucleotides of the template.

21. The method according to claim 20, wherein the DNA polymerase is a modified *Bacillus stearothermophilus*, *Bacillus caldotenax* or *Bacillus caldolyticus* DNA polymerase.

22. The method according to claim 21, wherein the DNA polymerase has the amino acid sequence of SEQ ID NO:4.

23. The method according to claim 21, wherein the DNA polymerase is encoded by a DNA segment having the nucleotide sequence of SEQ ID: NO 3.

24. A method for producing a modified form of a DNA polymerase which during DNA sequencing selectively discriminates against incorporation of fluorescent dye-labeled dideoxynucleotide terminators ddCTP and ddATP but does not discriminate against incorporation of fluorescent dye-labeled dideoxynucleotide terminators ddTTP and ddGTP, comprising the step of

modifying a DNA polymerase which has an amino acid sequence that shares not less than 95% homology of a DNA polymerase isolated from a

strain of *Bacillus stearothermophilus*, *Bacillus caldotenax* or *Bacillus caldolyticus*, so that the modified DNA polymerase includes threonine, proline and leucine at positions 342-344, respectively, and tyrosine at position 422.

25. The method according to claim 24, wherein the DNA polymerase has proofreading 3'-5' exonuclease activity during DNA sequencing of a DNA strand from a template, such that the DNA polymerase functions to excise mismatched nucleotides from the 3' terminus of the DNA strand at a faster rate than the rate at which the DNA polymerase functions to remove nucleotides matched correctly with nucleotides of the template.

26. The method according to claim 24, wherein the DNA polymerase has the amino acid sequence of SEQ ID:NO 4.

27. The method according to claim 24, wherein the DNA polymerase is encoded by a DNA segment having the nucleotide sequence of SEQ ID:NO 3.

28. A method for producing a modified form of a DNA polymerase which during DNA sequencing selectively discriminates against incorporation of fluorescent dye-labeled dideoxynucleotide terminators ddCTP and ddATP but does not discriminate against incorporation of fluorescent dye-labeled dideoxynucleotide terminators ddTTP and ddGTP, comprising the step of

modifying a nucleotide sequence encoding a DNA polymerase which has an amino acid sequence that shares not less than 95% homology of a DNA

polymerase isolated from a strain of *Bacillus stearothermophilus* or *Bacillus caldotenax*, so that the modified nucleotide sequence encodes threonine, proline and leucine residues at positions 342-344, respectively, and a tyrosine residue at position 422.

29. A DNA construct comprising:

(i) a nucleotide sequence encoding a DNA polymerase which has an amino acid sequence that shares not less than 95% homology of a DNA polymerase isolated from a strain of *Bacillus stearothermophilus* or *Bacillus caldotenax*, which nucleotide sequence encodes threonine, proline and leucine residues at positions 342-344, respectively, and a tyrosine residue at position 422; and

(ii) a vector, for introducing the DNA construct into euaryotic or procaryotic host cells.

30. The DNA construct according to claim 29 wherein the vector is a cloning vector or an expression vector.

31. A kit for direct DNA sequencing comprising the modified DNA polymerase of claim 1 and at least one fluorescent dye-labeled ddNTP.

32. The kit according to claim 31 wherein the modified DNA polymerase is a modified *Bacillus stearothermophilus*, *Bacillus caldotenax* or *Bacillus caldolyticus* DNA polymerase.

33. The kit according to claim 31 wherein the modified DNA polymerase has an amino acid sequence that shares not less than 95%

homology of a DNA polymerase isolated from a strain of *Bacillus stearothermophilus*, *Bacillus caldotenax* or *Bacillus caldolyticus*.

34. The kit according to claim 31 wherein the modified DNA polymerase is modified DNA polymerase obtained from a mesophilic bacterium.

35. The kit according to claim 31 wherein the modified DNA polymerase has proofreading 3'-5' exonuclease activity during DNA sequencing of a DNA strand from a template, such that the DNA polymerase functions to excise mismatched nucleotides from the 3' terminus of the DNA strand at a faster rate than the rate at which the DNA polymerase functions to remove nucleotides matched correctly with nucleotides of the template.

36. The kit according to claim 31, wherein the modified DNA polymerase is modified *Bacillus stearothermophilus* DNA polymerase which has proofreading 3'-5' exonuclease activity during DNA sequencing of a DNA strand from a template, such that the DNA polymerase functions to excise mismatched nucleotides from the 3' terminus of the DNA strand at a faster rate than the rate at which the DNA polymerase functions to remove nucleotides matched correctly with nucleotides of the template.

37. The kit according to claim 31 wherein the modified DNA polymerase has the amino acid sequence SEQ ID NO:4.

38. The kit according to claim 31



wherein the modified DNA polymerase is encoded by a DNA segment having the nucleotide sequence of SEQ ID:NO 3.

39. A modified nucleotide sequence encoding a DNA polymerase which has an amino acid sequence that shares not less than 95% homology of a DNA polymerase isolated from a strain of *Bacillus stearothermophilus* or *Bacillus caldotenax*, which nucleotide sequence encodes threonine, proline and leucine residues at positions 342-344, respectively, and a tyrosine residue at position 422.

40. The modified nucleotide sequence according to claim 39 which has the nucleotide sequence of SEQ ID:NO 3.

41. A method of dye-labeled primer sequencing of a DNA strand comprising the steps of:

- i) hybridizing a fluorescent dye-labeled primer to a DNA template to be sequenced;
- ii) extending the primer using the modified DNA polymerase of claim 1, in the presence of adequate amounts of nucleotide bases dATP, dGTP, dCTP and dTTP, or their analogs, and dideoxynucleotide terminators,

under such conditions that the DNA template is sequenced.

42. The method according to claim 41, wherein the DNA polymerase is a modified *Bacillus stearothermophilus* DNA polymerase.

43. The method according to claim 41, wherein the modified DNA polymerase has an amino

acid sequence that shares not less than 95% homology of a DNA polymerase isolated from a strain of *Bacillus stearothermophilus*, *Bacillus caldotenax* or *Bacillus caldolyticus*.

44. The method according to claim 41, wherein the modified DNA polymerase has the amino acid sequence SEQ ID NO:4.

45. The method according to claim 41, wherein the DNA polymerase is encoded by the nucleotide sequence SEQ ID NO:3.

46. The method according to claim 41, wherein the DNA polymerase is a modified DNA polymerase obtained from a mesophilic bacterium.

47. The method according to claim 41, wherein the DNA polymerase is a thermostable DNA polymerase having proofreading 3'-5' exonuclease activity during DNA sequencing of a DNA strand from a template, such that the DNA polymerase functions to excise mismatched nucleotides from the 3' terminus of the DNA strand at a faster rate than the rate at which the DNA polymerase functions to remove nucleotides matched correctly with nucleotides of the template.

48. The method according to claim 47, wherein the DNA polymerase is a modified *Bacillus stearothermophilus*, *Bacillus caldotenax* or *Bacillus caldolyticus* DNA polymerase.

49. A method of radioisotope-labeled sequencing of a DNA strand comprising the steps of:

- i) providing four separate tubes, each

containing DNA template to be sequenced, a primer, dATP, dTTP, dCTP and dGTP, wherein one of dATP, dTTP, dCTP or dGTP is labeled with a radioisotope, and DNA polymerase according to claim 1,

wherein each tube contains an appropriate amount of one of ddATP, ddTTP, ddCTP or ddGTP;

ii) allowing DNA sequencing of the DNA template to be carried out; and

iii) determining the sequence of the DNA template.

50. The method according to claim 49, wherein the DNA polymerase is a modified *Bacillus stearothermophilus* DNA polymerase.

51. The method according to claim 49, wherein the modified DNA polymerase has an amino acid sequence that shares not less than 95% homology of a DNA polymerase isolated from a strain of *Bacillus stearothermophilus*, *Bacillus caldotenax* or *Bacillus caldolyticus*.

52. The method according to claim 49, wherein the modified DNA polymerase has the amino acid sequence SEQ ID NO:4.

53. The method according to claim 49, wherein the DNA polymerase is encoded by the nucleotide sequence SEQ ID NO:3.

54. The method according to claim 49, wherein the DNA polymerase is a modified DNA polymerase obtained from a mesophilic bacterium.

55. The method according to claim 49, wherein the DNA polymerase is a thermostable DNA polymerase having proofreading 3'-5' exonuclease

activity during DNA sequencing of a DNA strand from a template, such that the DNA polymerase functions to excise mismatched nucleotides from the 3' terminus of the DNA strand at a faster rate than the rate at which the DNA polymerase functions to remove nucleotides matched correctly with nucleotides of the template.

56. The method according to claim 55, wherein the DNA polymerase is a modified *Bacillus stearothermophilus*, *Bacillus caldotenax* or *Bacillus caldolyticus* DNA polymerase.